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Developmental Exposure to 4-hydroxy-2,3,3′,4′, 5-pentachlorobiphenyl (4-OH-CB107): Long-Term Effects on Brain Development, Behavior, and Brain Stem Auditory Evoked Potentials in Rats

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In the present study the developmental neurotoxic effects of the PCB metabolite 4-OH-2,3,3′,4′,5-pentachlorobiphenyl (4-OH-CB107) were compared with effects caused by a mixture of parent polychlorinated biphenyl (PCB) congeners (Aroclor 1254). Pregnant female Wistar rats were exposed to 0.5 or 5 mg 4-OH-CB107, or 25 mg Aroclor 1254 per kg body weight from gestation days 10 to 16. Plasma thyroid hormone levels were significantly decreased in the offspring of all treatment groups at postnatal day 4 (PND 4). Behavioral experiments using an open field paradigm revealed an impaired habituation in male offspring of all treatment groups at PND 130. Passive avoidance experiments indicated significant influences on the time course of step-down latencies across trials in exposed male rats. Catalepsy induced by haloperidol showed increases in latencies to movement onset in female offspring exposed to 0.5 mg 4-OH-CB107 compared to Aroclor 1254 treated offspring at PND 168–175. Male offspring exposed to 4-OH-CB107 or Aroclor 1254 showed decreases in latencies compared to control animals. Brain stem auditory evoked potentials (BAEPs) measured at PND 300–310 showed significant increases in auditory thresholds in the low frequency range between Aroclor 1254 and 4-OH-CB107 (5 mg/kg bw) treated animals. Measurements of neurotransmitter levels revealed effects of Aroclor 154 exposure on both the dopaminergic and the serotonergic systems, whereas 4-OH-CB107 exposure affected dopaminergic and noradrenergic systems, with slight but not significant effects on the serotonergic system. These results indicate that 4-OH-CB107 is able to induce long-term effects on behavior and neurodevelopment. The observed effects for 4-OH-CB107 are similar to, but in some aspects different from, the effects observed after Aroclor 1254 exposure.

Key Words: **PCB, metabalite, BAEP, neurotransmitters.**

INTRODUCTION

In utero and lactational exposure to polychlorinated biphenyls (PCBs) may result in developmental effects in the offspring of laboratory animals (reviewed by Brouwer *et al*., 1995). These developmental effects include alterations in thyroid hormone homeostasis (Brouwer *et al*., 1998; Brucker-Davis, 1998; Morse *et al*., 1996a), neurobehavioral effects (Lilienthal *et al*., 1997; Schantz *et al*., 1995; Seo *et al*., 1995; Tilson and Kodavanti, 1997), reproductive and endocrine effects (Hany *et al*., 1999a; Peterson *et al*., 1993) and neurochemical effects (Morse *et al*., 1996b; Seegal, 1996). The adverse effects caused by PCBs are dependent on the time of exposure and the structural characteristics of the PCB congener. For example, *ortho*-substituted PCB congeners reduce brain dopamine concentrations in both adult rats and rats exposed *in utero* through weaning, whereas coplanar, dioxin-like PCB congeners affect neurotransmitter levels predominantly after *in utero* exposure (Seegal *et al*., 1996). It is postulated that the changes in neurochemical parameters in PCB-exposed offspring may be causatively linked to the observed neurobehavioral changes such as locomotor activity, delayed spatial learning, and active and passive avoidance (Schantz *et al*., 1995; Seegal *et al*., 1996; Seo *et al*., 1995). The mechanism by which PCBs interfere with neuronal development causing long-term effects on neurobehavior is unknown, but PCB-induced hypothyroidism (reviewed in Brouwer *et al*., 1998), as well as the observed changes in neurotransmitter levels, are both suggested to play a role. Perinatal exposure to Aroclor 1254 is known to reduce fetal and neonatal thyroid hormone levels in rats (Morse *et al*., 1996a). Long-term effects observed in offspring exposed perinatally to Aroclor 1254 are, *e.g.*, alterations in serotonin metabolism in several brain areas (Morse *et al*., 1996b) and a selective low-frequency hearing loss (Goldey *et al*., 1995a). Goldey and Crofton (1998) showed that this hearing loss could be partially overcome by T_4 replacement.

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Previous studies at our laboratory have shown that exposure of pregnant rats to Aroclor 1254 from gestational days (GD) 10 to 16 resulted in a substantial accumulation of mainly one hydroxylated metabolite (2,3,3′,4′,5-pentachlorobiphenyl, 4-OH-CB107) in the fetal compartment, especially in the brain (Morse *et al*., 1996a). This PCB metabolite is one of the major metabolites identified in blood samples of seals, rats, and humans (Bergman *et al*., 1994; Sjödin *et al*., 2000). 4-OH-CB107 is a metabolite of 2,3,3′,4,4′-pentachlorobiphenyl (CB-105) and of 2,3′,4,4′,5-pentachlorobiphenyl (CB-118) (Sjödin *et al*., 1998). The presence of 4-OH-CB107 in blood plasma of humans and wildlife, its observed accumulation in brain from animals exposed to Aroclor 1254, and its potency to induce drastic decreases in thyroid hormone levels after prenatal exposure (Meerts *et al*., 2002), all prompted us to investigate the potency of 4-OH-CB107 to induce possible neurobehavioral and neurochemical changes in rat offspring exposed *in utero*. For this purpose, pregnant rats were exposed to 5 and 0.5 mg 4-OH-CB107 per kg bw per day from GD 10 to GD 16. Exposure of pregnant rats to a concentration of 5 mg 4-OH-CB-107 per kg bw per day from GD 10 to GD 16 resulted in a concentration of 4-OH-CB107 in the fetal compartment comparable to the concentration that was reached after *in utero* exposure to Aroclor 1254 (Meerts *et al*., 2002; Morse *et al*, 1996a). In fetal plasma at GD 20, concentrations of 37.2 ± 5.14 nmol/ml were measured after *in utero* exposure to 4-OH-CB107 (Meerts *et al*., 2002). A concentration of 0.5 mg/kg bw per day was additionally chosen to evaluate possible dose-related effects of 4-OH-CB-107. To determine if the effects observed in offspring exposed to Aroclor 1254 (as described by Morse *et al*., 1996a) can be explained mainly by the accumulation of the metabolite 4-OH-CB107 in fetuses and neonates, an additional group of rats was exposed to Aroclor 1254.

ANIMALS, MATERIALS, AND METHODS

Chemicals. 4-Hydroxy-2,3,3′,4′,5-pentachlorobiphenyl (4-OH-CB107) was synthesized as described by Bergman *et al*. (1995) and was at least 99.9% pure. Aroclor 1254 was kindly donated by Prof. Dr. M. van den Berg (IRAS, University of Utrecht, the Netherlands).

Animals and treatment. All experimental procedures involving animals were approved by the Animal Welfare Committee of Wageningen University. Wistar WU rats (60 females, 30 males; 14 weeks old) were purchased from Charles River (Sulzfeld, Germany) and allowed to acclimatize for 3 weeks. The rats were maintained in Macrolon cages in rooms with 50 ± 10% humidity and 21° ± 2°C, in an artificial 12 h:12 h light–dark cycle with lights on at 06:00 h. Rat chow (Hope Farms, Woerden, the Netherlands) and tap water were supplied *ad libitum*. After the acclimatization period two females were placed in a cage with one male overnight from 17:00 to 8:00 h. The females were examined each morning for copulation by checking for the presence of sperm in the vaginal smear. When spermatozoa were found, that day was designated as day 0 of gestation (GD 0) and females were housed individually. On GD 10 the pregnant rats were divided randomly into the different treatment groups and transferred to a Macrolon, stainless-steel cage to facilitate the collection of PCB-contaminated feces.

Pregnant females (13 per exposure group) received a daily oral dose of 0, 0.5 or 5 mg 4-OH-CB107 per kg body weight, dissolved in corn oil (2 ml/kg body weight) from GD 10–16. For comparison of the effects of the PCB metabolite with effects caused by parent PCB congeners, a fourth group of rats was dosed with 25 mg Aroclor 1254 per kg body weight from GD 10 to GD 16. That dose level was chosen because 25 mg/kg Aroclor 1254 given to pregnant rats gave rise to a PCB metabolite production equivalent to 5 mg/kg of 4-OH-CB107 (Morse *et al*., 1996a). Maternal body weights were monitored daily throughout gestation and lactation. On GD 20, pregnant females were transferred to bedding material and were given paper tissues with which to make a liter. The offspring were counted, inspected for signs of overt toxicity, and weighed at birth (postnatal day [PND] 0), and again on PNDs 1, 4, 7, 14, and 21. After weaning, body weights of the offspring were monitored weekly until sacrifice. On PND 4, litters were standardized to 4 males and 4 females. Generally, this required culling of excess offspring. However, in a few cases the standardized litter required pups from two dams. To maintain litter independence, no dam was allowed to contribute pups to more than one litter. In addition, pups transferred from one litter to another were not used for any analysis. The standardized litter became the experimental unit, and all treatment mean values reported are litter based. The pups were numerically marked on their feet to identify individual animals within a litter. Excess pups were decapitated at PND 4, and trunk blood was collected for thyroid hormone analysis. Liver, kidneys, brain, and thymus were weighed.

After weaning at PND 21, pups were housed with littermates in unisexual groups, two pups per cage. After puberty, the offspring were split into two cohorts. One cohort (n = 41 litters with 2 males and 2 females per litter) was used for examining reproductive effects (described separately). From the other cohort (also 41 litters with 2 males and 2 females per litter) were housed in unisexual groups. At least 2 weeks before the onset of behavioral testing, animals were housed individually in unisexual groups.

Thyroid hormone analysis. Plasma total T_4 (TT_4), free T_4 (FT_4) and total T_3 (TT_3) were analyzed in duplicate using chemiluminescence kits and plasma thyroid stimulating hormone (TSH) concentrations were analyzed with a specific rat TSH immunoassay. All kits were purchased from Amersham (Amersham, Buckinghamshire, UK). The intra- and interassay variations were below 10% for all hormones.

Behavioral testing. Behavioral tests were conducted in naive male and female offspring. Only one randomly selected male and female rat per litter was used for one behavioral test. The experiments were performed in a blind fashion.

At PND 130, locomotor activity of male and female offspring was measured in an open field paradigm, following the procedures described by Hany *et al*. (1999a). Briefly, 8 males and 8 females per exposure group (from different litters) were placed in a white octagonal arena with a diameter of 75 cm for 9 min, subdivided into three intervals of three minutes each. The open field was evenly illuminated by indirect light provided by two lamps (40 W each). The movements of the animals were recorded by a video camera, which was connected to a digital image processing system (Ethovision, Noldus, Wageningen, the Netherlands). The plane of the open field was subdivided in an inner zone, measuring 50 cm in diameter, and an outer zone, consisting of the remaining outer ring.

The passive avoidance behavior was studied in a step-down task at PND 130 as described in detail by Weinand-Härer *et al*. (1997). In short, a 1 mA footshock of 1 s duration was used in the conditioning trial. Subsequently, step-down latencies from a platform were tested 5 min, 4 h, and 24 h after the conditioning, with a maximal duration of 180 s per trial.

Between PNDs 168 and 175, catalepsy induced by the dopamine receptor blocker haloperidol was tested in male and female offspring as described by Weinand-Härer *et al*. (1997). Only females in the diestrous stage of the estrous cycle were used for the test. Haloperidol was injected intraperitoneally at a concentration of 0.3 mg/kg body weight, and the animals were tested 30 and 60 min after injections by placing the rat in three postures: (1) placing both front paws on a horizontal bar 9 cm above the surface, (2) putting the rat on a grid, with a 10 degrees deviation from the vertical plane, and (3) placing the paws in four different holes of a box. Time for retraction of the first paw and descent latency, latency to movement onset, and retraction of a front leg and a hind leg were

determined on conditions (1), (2), and (3), respectively. If the rat failed to move one paw, testing was terminated at 180 s on all conditions.

Brain stem auditory evoked potentials. Between PND 300 and PND 310 auditory thresholds and peak latencies were studied in male and female offspring by recording BAEPs according to methods adapted from Lilienthal and Winneke (1991). The animals were housed individually for the 2 weeks before the start of the BAEP measurements. The animals were sedated with an i.p. injection of ketamine (90 mg/kg body weight for males, 55 mg/kg body weight for females) and maintained on xylazine (3.5 mg/per kg body weight for males, and 3 mg/kg body weight for females). Rats were placed on a heating pad to prevent cooling. Needle electrodes were placed under the skin at the vertex and behind both ears. The ground electrode was contralateral to the stimulated right ear. The left ear was occluded by a tissue plug in the outer ear channel. Impedance was 5 kΩ at the maximum.

Brain stem auditory evoked potentials were recorded on a Pathfinder II (Nicolet Inc., Madison, WI) after stimulation with rarefaction clicks using a shielded high-frequency piezo loudspeaker and a SM 700 multisignal auditory generator. Clicks were presented at seven different sound pressure levels (72, 62, 52, 42, 32, 22, and 12 dB, re. 20 μPa) using a repetition rate of 11.1 Hz. The pulse width was set to 50 μs. In addition, BAEPs evoked by tone pips at different frequencies (20, 16, 8, 4, 2, 1, and 0.5 kHz) were recorded at sound pressure levels (SPL) ranging from 88 to 8 dB. Because of the general lower hearing capacity at the lower frequency border in rats, higher sound pressure levels, up to 110 dB, were used at 0.5 kHz for threshold determination. Tone pips with frequencies below 4 kHz were delivered by shielded TDH 39P earphones. Sound pressure levels were calibrated with a precision sound level meter (type 2230, Brüel & Kjaer) equipped with a 0.5″ condenser microphone (type 4165, Brüel & Kjaer). The whole set-up was calibrated using a pistonphon (type 4220, Brüel & Kjaer).

For recording, the sweep duration was set at 8 ms. Sweeps were sampled with a rate of 62.5 kHz. For each BAEP, 1000 sweeps were averaged using the artifact rejection. For the determination of thresholds, sound pressure level was progressively lowered until even the most prominent peak, no. II, was no longer identified in the BAEP.

Dissections. Dams were sacrificed at weaning (PND 21), and blood was collected via the *vena cava* in heparinized tubes for thyroid hormone measurements. Liver, kidneys, adrenals, thymus, brain, spleen, uterus, and ovary were collected, blotted dry, and weighed.

Male and female offspring were sacrificed between PND 310 and PND 325. The animals were killed by decapitation within 15 s of removal from their cages. Dissections were conducted between 08:00 and 12:00. Trunk blood was collected in Eppendorf tubes (for serum preparation) and heparinized tubes (for plasma) for hormone analysis. Brains were dissected rapidly on ice (within 5 min) and separated into the following regions: lateral olfactory tract, prefrontal cortex, frontal cortex, caudate nucleus, nucleus accumbens. Brain regions were weighed, immediately frozen in liquid nitrogen, and stored at −80°C until analysis of biogenic amines.

Measurement of biogenic amines. Brain regions were thawed by the addition of 10 volumes of ice-cold 0.2 N perchloric acid containing 100 mg/l of ethylene glycol-bis-(B-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) and homogenized on ice with an ultrasonic tissue disruptor (Vibra cell, Sonics& Materials Inc. Danbury, CT) for 30 s. The regional brain levels of the neurotransmitters dopamine (DA), 5-hydroxytryptamine (5-HT), and norepinephrine (NE), as well as the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and the 5-HT metabolite 5-hydroxyindole-3-acetic acid (5-HIAA), were determined by high-performance liquid chromatography (HPLC) separation and electrochemical analysis as described elsewhere (Seegal *et al*., 1986).

Statistical analysis. Statistical analyses were performed using the SPSS or SAS statistical software package. Depending on the data structure, different statistical analyses were conducted. The data of the open-field and passive avoidance test were assessed by analysis of variance (ANOVA), with repeated measures on the factor time. Wilks's lambda was used to analyze within subjects effects. The catalepsy data were analyzed with the median test (Siegel, 1952). Brain stem auditory evoked potential results were analyzed using multivariate analysis of variance (MANOVA) with repeated measures on the factors sound pressure level (SPL) and rate. Sex was included as an independent factor, together with treatment, in the multivariate analysis. In addition, preplanned univariate ANOVAs with repeated measures on one factor, SPL or rate, were calculated, and separate ANOVAs were conducted for each sex. Following significant overall F-tests post hoc comparison of group means was performed using the Ryan-Einot-Gabriel-Welsch multiple range test.

Data on regional brain biogenic amine levels and thyroid hormone levels were evaluated with one-way analysis of variance (ANOVA). Levene's test was used to evaluate homogeneity of variances, and the Bonferroni test was used to compare individual treatment means when ANOVA indicated that significant differences were present. When the Levene's test was significant, a log transformation of the data was performed prior to ANOVA.

RESULTS

Body and Organ Weights

No effects were observed on maternal body weight gain, mean and total fetal body weight, number of implantation sites, resorptions, number of fetuses per litter, or sex ratio following prenatal exposure to 4-OH-CB107 or Aroclor 1254 from GD 10–16 (data not shown). Developmental landmarks (*i.e.*, pinna detachment, age at eye opening, anogenital distance, crown–rump length, age at vaginal opening, and preputial separation), estrous cyclicity and F_1 reproduction effects observed after 4-OH-CB107 or Aroclor 1254 exposure are reported elsewhere (Meerts *et al*., 2004).

Plasma Thyroid Hormone Levels

Plasma thyroid hormone and thyroid stimulating hormone (TSH) levels from 4-OH-CB107 or Aroclor 1254 exposed dams showed no significant differences relative to controls at 21 days postpartum (data not shown). However, 4 days after birth (PND 4), male and female neonatal total thyroxine (TT_4) levels were significantly decreased in all exposure groups (Fig. 1). The decrease in TT_4 levels was highest in the Aroclor 1254 exposed group (66% and 42% decrease in male and female offspring, respectively, compared to control animals). Plasma FT_4 levels at PND 4 were lower (though not significantly) in 4-OH-CB107 exposed male offspring than in controls (Fig. 2A). Aroclor 1254 exposed male offspring also showed a decrease in FT_4 levels, but this was not significant, possibly because of the low number of animals in this group ($n = 6$). In female offspring, the effects on FT_4 levels were less pronounced (Fig. 2B).

Plasma TT_3 levels at PND 4 were decreased in both male and female offspring of the Aroclor 1254 exposed animals, but this reduction was only significant in males (Fig. 3). No decreases in TT_3 levels were observed in offspring exposed to 4-OH-CB107. In addition, neonatal TSH levels were not affected at PND 4 in both male and female offspring of the different treatment groups (data not shown).

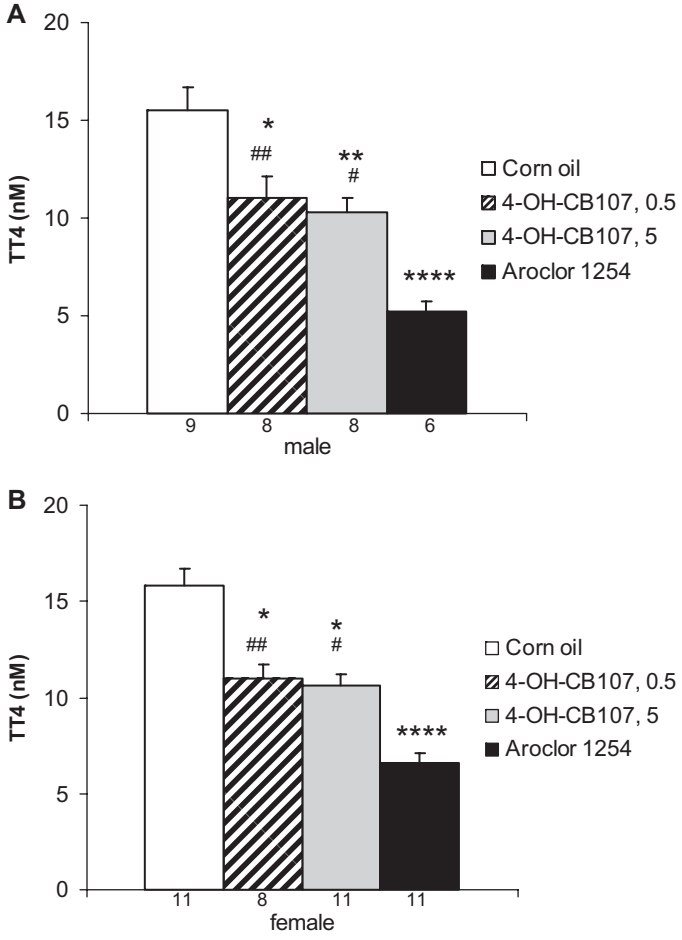

FIG. 1. Total thyroxine (TT_4) levels in male (A) and female (B) offspring at postnatal day 4 (PND 4) following maternal exposure to corn oil, 4-OH-CB107 (PCB-OH, 0.5 or 5 mg-kg) or Aroclor 1254. #Significant differences from Aroclor 1254 ($p < 0.05$); ##($p < 0.01$);. *Significant differences from control ($p < 0.05$); **$p < 0.01$; ***$p < 0.005$; ****$p < 0.001$. The number of different litters (n) is given at the base of each column.

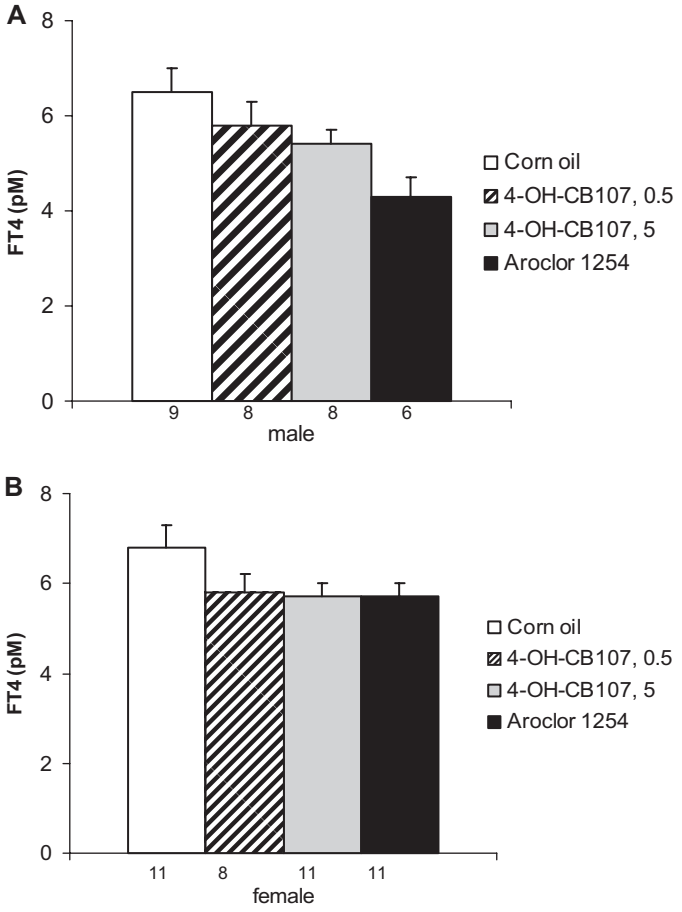

FIG. 2. Free thyroxine (FT_4) levels in male (A) and female (B) offspring at postnatal day 4 (PND 4) following maternal exposure to corn oil, 4-OH-CB107 (PCB-OH, 0.5 or 5 mg-kg), or Aroclor 1254. The number of different litters (n) is given at the base of each column.

At 11 months of age, no differences could be observed in thyroid hormone or TSH levels in male and female offspring (data not shown).

Behavioral Tests

Locomotor activity. In male offspring, all exposed groups showed a significantly higher overall locomotor activity in the open field paradigm in the last 3 min compared to controls (Fig. 4B). There was a significant interaction between exposure and time [$F(6,52) = 3.37$; $p < 0.05$] as well as a significant quadratic contrast for exposure [$F(3,27) = 3.52$; $p < 0.05$], indicating a different time course of activity in different treatment groups. *Post hoc* testing revealed significant elevations of locomotor activity in all exposed groups in comparison to controls during the last 3 min of the measuring period ($p <$ 0.05). No effects could be seen in female offspring (Fig. 4A). There was also no exposure-related difference in the preference for the outer or inner zone in both sexes (data not shown).

Passive avoidance. No exposure-related differences were observed in avoidance latencies in female rats at PND 130. Data indicated a reduction of latencies 4 h after the conditioning trial in male rats of the low-dose 4-OH-CB107 group, but not in the high-dose group (Fig. 5A). According to ANOVA with repeated measures, there was a significant interaction between exposure and time [$F(6,52) = 2.24$; $p \leqslant 0.05$] and a significant quadratic contrast, illustrating exposure-related differences in the course of latencies across the trials [$F(3,27) = 4.98$; $p <$ 0.05]. In addition, the reduction of latencies in the low-dose 4-OH-CB107 group was significant in comparison to controls and the high-dose group 4 h after the conditioning trial according to *post hoc* testing ($p < 0.05$). To verify these subtle changes, the measurements were repeated by testing naive littermates at PND 290 (Fig. 5B). Again, a significant interaction between time and exposure was detected [$F(6,54) = 2.47$; $p < 0.05$],

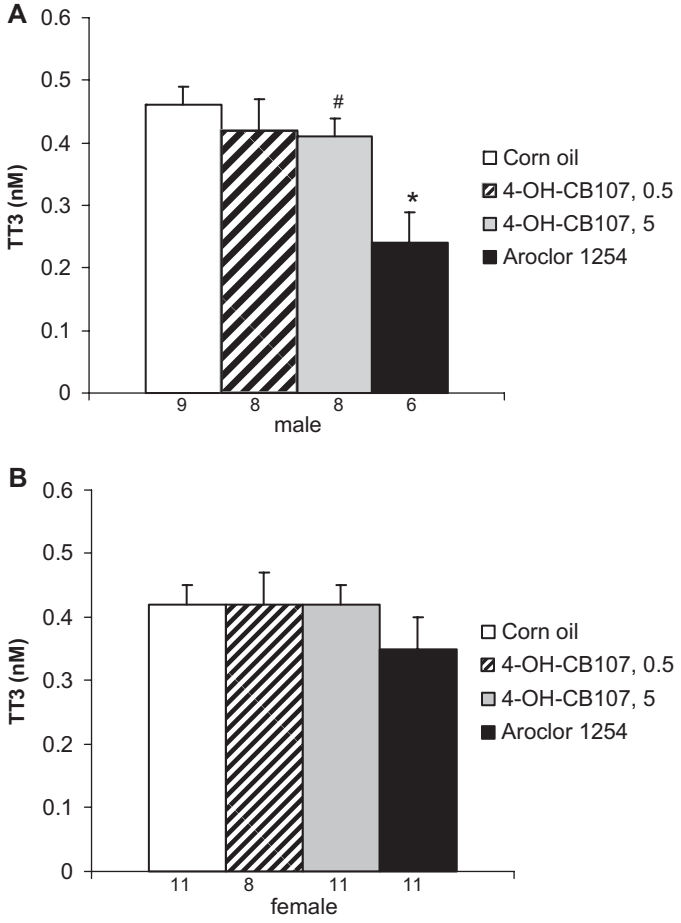

FIG. 3. Total triiodothyronine (TT_3) levels in male (A) and female (B) offspring at postnatal day 4 (PND 4) following maternal exposure to corn oil, 4-OH-CB107 (PCB-OH, 0.5 or 5 mg-kg) or Aroclor 1254. * = significantly different from control ($p < 0.05$); # = significantly different from Aroclor 1254 ($p < 0.05$). The number of different litters (n) is given at the base of each column.

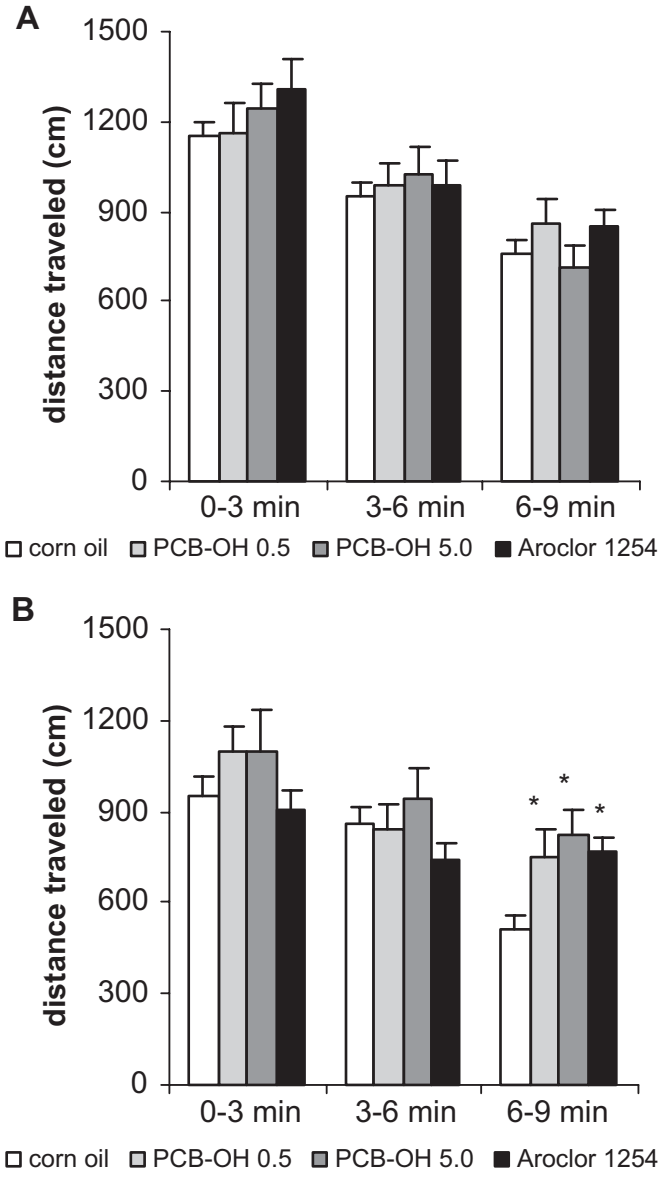

FIG. 4. Total distance (in cm) traveled by female (A) and male (B) rats in the whole arena of the open field. * = significantly different from control ($p < 0.05$). PCB-OH = 4-OH-CB107. Total number of litters per exposure group was eight, except for male offspring exposed to 0.5 mg/kg 4-OH-CB107 ($n = 7$).

as was a significant linear contrast [$F(3,28) = 2.97$; $p < 0.05$]. A steady increase in latencies across the trials could be observed only in the Aroclor 1254 group, whereas a plateau was reached in all other groups. These differences could not be attributed to different treatment groups according to *post hoc* tests.

Catalepsy. In males, significant differences between groups were detected in the latency to move a front paw on the grid (Fig. 6A and 6B). Sixty minutes after haloperidol injection, the time needed to move the front paw was significantly reduced in rats treated with the low dose of 4-OH-CB107 (0.5 mg/kg; $p < 0.05$) and rats treated with Aroclor 1254 ($p < 0.01$) compared to controls. The values of the high-dose group of 4-OH-CB107 were changed in the same direction, though not significantly. No significant treatment-related effects were detected in males on the bar or the box (data not shown).

In females, a marginally significant increase ($p < 0.1$) was observed in the latency to remove a front paw on the grid in the low-dose 4-OH-CB107 group compared to controls 30 min after haloperidol injection (Fig. 7A). In Aroclor 1254 treated females, this increase was more pronounced ($p < 0.05$). Sixty minutes after haloperidol injection, the latency was marginally reduced ($p < 0.1$) in the low-dose 4-OH-CB107 group compared to the high-dose group (Fig. 7B).

Latencies to retract a front leg or a hind leg from the box in females 30 min after haloperidol injection were significantly increased in the low-dose PCB-OH group compared to Aroclor 1254 treated females (data not shown).

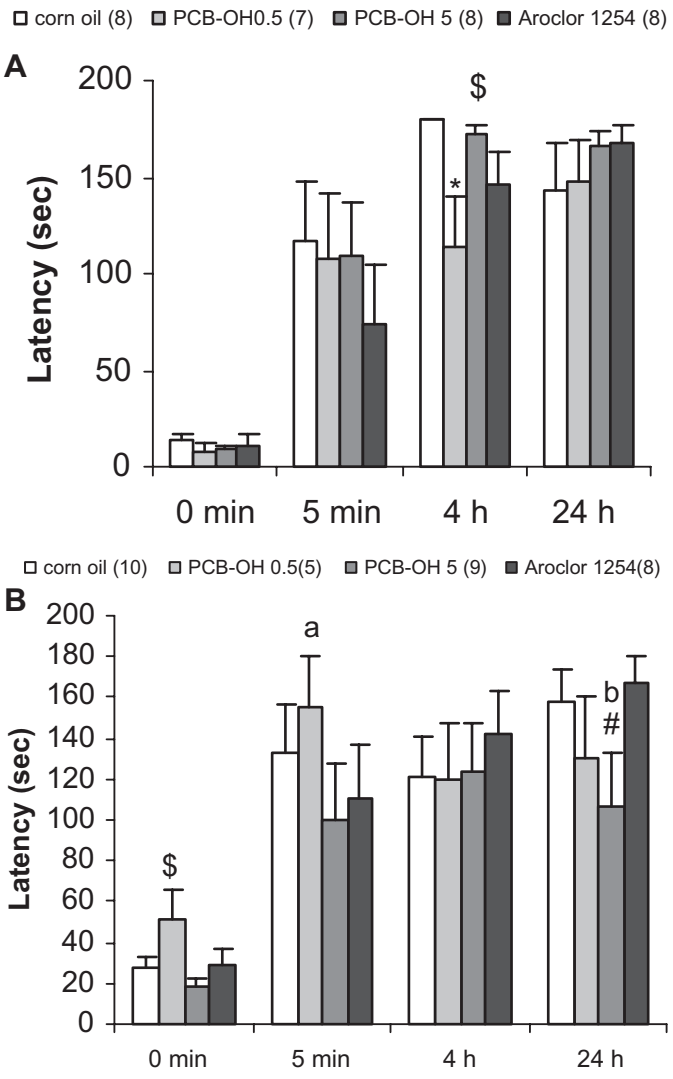

FIG. 5. Latencies (in seconds) in the passive avoidance task from naive males at PND 130 (A) and PND 290 (B). Numbers in parentheses indicate the number of litters per exposure group. *a* = marginally different from 4-OH-CB107 5 mg/kg; $p < 0.1$; *b* = marginally different from corn oil; $p < 0.1$; # = significantly different from Aroclor 1254; $p < 0.05$; $ = significantly different from 4-OH-CB107 5 mg/kg; $p < 0.05$. PCB-OH = 4-OH-CB107.

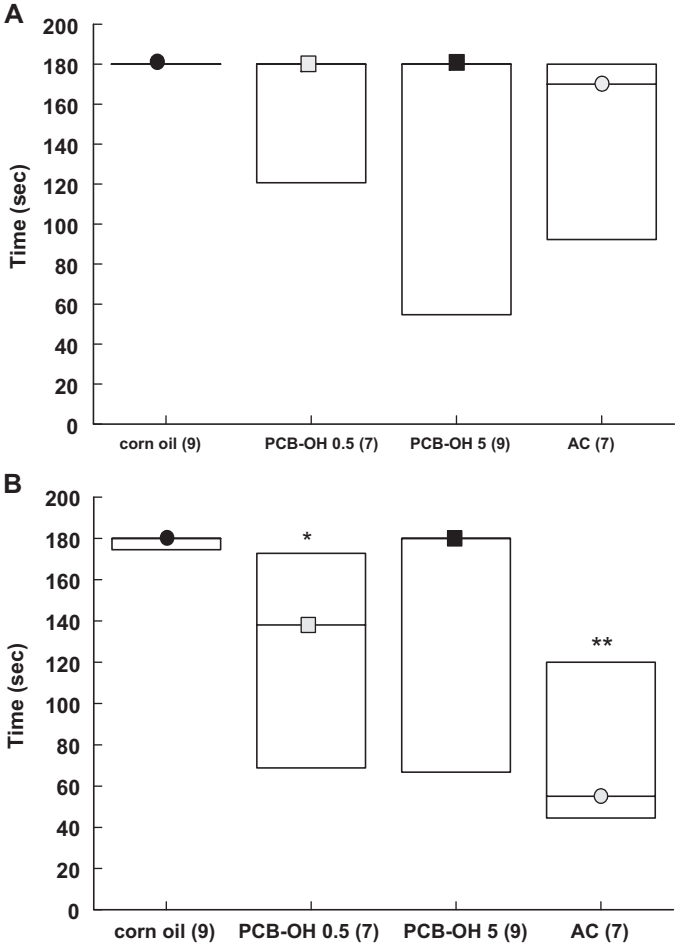

FIG. 6. Latency to move a front paw on the grid in males, 30 min (A) and 60 min (B) after the challenge with haloperidol. Median, 25% and 75% quartiles are presented. Numbers in parentheses indicate the number of litters per exposure group. Significant difference to control group: $*p < 0.05$; $**p < 0.01$. PCB-OH = 4-OH-CB107; AC = Aroclor 1254.

Brain Stem Auditory Evoked Potentials

Auditory thresholds. For the tone pips, significant treatment-related influences on auditory thresholds were observed at 0.5 kHz [$F(3,56) = 4.64$; $p < 0.05$] and 2 kHz [$F(3,56) = 2.87$; $p < 0.05$] in a two-way ANOVA with sex and treatment as independent factors. Sex exerted a significant effect at 0.5 kHz ($p < 0.05$), but not at 2 kHz ($p < 0.1$).

In females, auditory thresholds were significantly affected by exposure at 1 kHz [$F(3,34) = 3.39$; $p < .05$)]. Thresholds were elevated in Aroclor 1254 exposed females compared to all other groups, the mean increases to controls measuring 1.7, 4.7, and 1.7 dB at 0.5, 1, and 2 kHz, respectively; however, statistical significance (*post hoc* test, $p < 0.05$) was obtained only for the difference between Aroclor 1254 treated females and female rats exposed to 5 mg/kg 4-OH-CB107 (Fig. 8). This outcome was supported by a significant interaction between frequency and treatment [$F(18,204) = 2.27$; $p < 0.01$] across all frequencies. There were no significant auditory threshold deficits in males (data not shown), only a marginally significant treatment effect at 500 Hz [$F(3,22) = 2.60$; $p < 0.08$].

With the exception of a significant overall sex effect [$F(1,58) = 4.44$; $p < 0.05$], no exposure-related effects were detected on click thresholds.

Peak latencies. Analysis of peak II at different frequencies and SPL revealed a significant interaction between SPL and treatment at the 500 Hz frequency in males [$F(12,84) = 2.43$; $p < 0.05$, data not shown]. Representative traces of BAEPs at 1 kHz and 88 dB are shown in Figure 9. Latency values of peak II at 1 kHz are given for three different sound pressure levels in

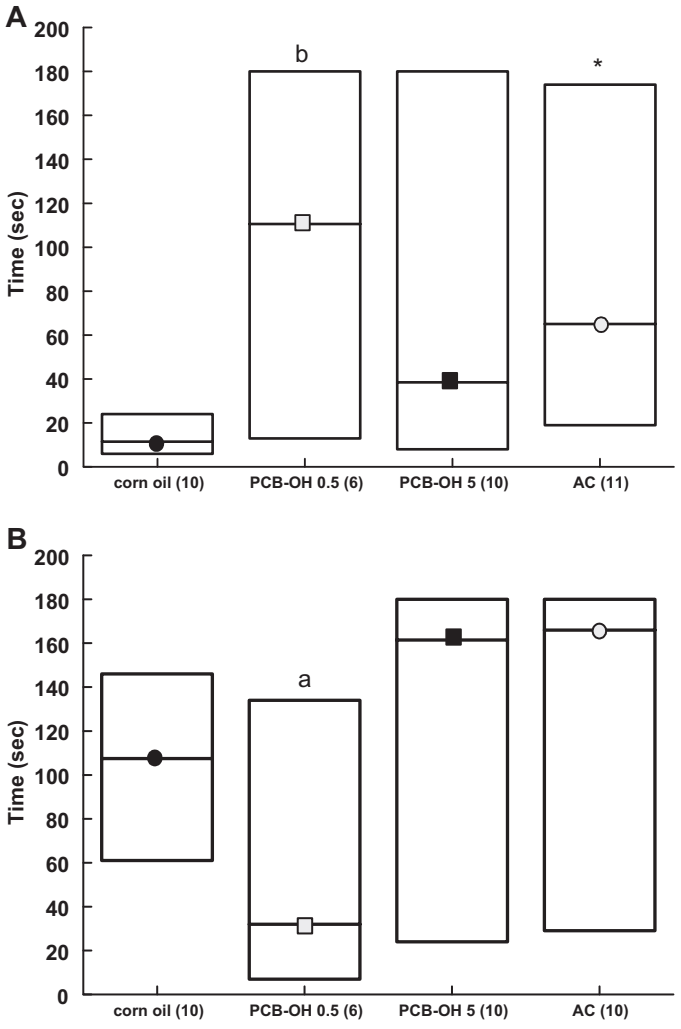

FIG. 7. Latency to move a front paw on the grid in females, 30 min (A) and 60 min (B) after the challenge with haloperidol. Median, 25% and 75% quartiles are presented. Numbers in parentheses indicate the number of litters per exposure group. *a* = marginally different from 4-OH-CB107 5 mg/kg; $p < 0.1$; *b* = marginally different from corn oil; $p < 0.1$. * = significantly different from corn oil, $p < 0.05$. PCB-OH = 4-OH-CB107; AC = Aroclor 1254.

Table 1. Compared to all other groups, male animals treated with 5 mg/kg 4-OH-CB107 exhibited the highest latency values at all frequencies and SPL tested. In addition, 4-OH-CB107–induced prolongation of peak II latency was dose-dependent at all levels used at 1 kHz. However, differences failed to reach statistical significance. Similar results were obtained for 2 kHz and 4 kHz (data not shown).

After click stimulation using different SPL, there was a significant main effect for exposure on peak II latency in both sexes [$F(3,54) = 3.06$; $p < 0.05$]. According to *post hoc* tests, no significant differences could be observed between sexes. Male rats exposed to 4-OH-CB107 exhibited dose-dependent increases in peak II latencies on all but the lowest SPL in comparison to controls and Aroclor 1254 treated rats. Representative BAEP traces for clicks at 72 dB are shown in Figure 10. According to *post hoc* tests, a significant difference was detected between males exposed to the low dose of 4-OH-CB107 and Aroclor 1254 treated animals at the lowest SPL ($p < 0.05$). All other differences were not significant. In females, dose-dependent increases in latencies due to metabolite exposure were observed only at the three lowest SPLs, but there were no significant differences between groups. Also, no significant differences were observed on latencies of peak IV and the interpeak latency between peaks II and IV (data not shown).

Biogenic amines. 3,4-Dihydroxyphenylacetic acid (DOPAC) levels were significantly decreased by 37% ($p < 0.05$) in the nucleus accumbens (NA) of male offspring exposed to 5 mg/kg 4-OH-CB107 compared to control animals (Table 2). In the caudate nucleus (CN), DOPAC levels were significantly lower in the 5 mg/kg 4-OH-CB107 group compared to Aroclor 1254 ($p < 0.01$), but not compared to controls. In females, a slight though not significant increase in DOPAC levels was observed in 4-OH-CB107 and Aroclor 1254 treated animals compared to controls in the NA, and a slight but not significant decrease in DOPAC levels in the CN of the 5 mg/kg 4-OH-CB107 dose group.

5-Hydroxyindole-3-acetic acid (5-HIAA) levels were significantly increased in the frontal cortex (FC) of Aroclor 1254 treated male offspring compared to controls (by 62.5%) and 4-OH-CB107 (by 50%) treated animals. The same trend, though not significant, is visible in female offspring. In the caudate nucleus (CN) 5-HIAA levels were significantly increased by 50% ($p < 0.01$) in males from the low 4-OH-CB107 group compared to Aroclor 1254 treated animals.

The concentrations of homovanillic acid (HVA) in the CN of male animals were significantly decreased by 22.5% ($p < 0.05$) in the high 4-OH-CB107 group compared to controls. No effects were observed on the ratio of DOPAC/DA and 5-HIAA/5-HT in different brain regions in both male and female offspring.

DISCUSSION

The purpose of the present study was to compare the possible developmental effects caused by the PCB-metabolite 4-hydroxy-2,3,3′,4′,5-pentaCB (4-OH-CB107) with parent compounds, using the commercial PCB mixture Aroclor 1254. The data obtained demonstrate that prenatal exposure to 4-OH-CB107 can induce adverse developmental neurotoxic effects on its own, which are similar, but also partly different from these caused by parent PCB congeners. Effects of the 4-OH-CB107 on developmental landmarks, steroid hormones, and female estrous cyclicity are described elsewhere (Meerts *et al.*, 2004).

Maternal exposure to 4-OH-CB107 or Aroclor 1254 resulted in a significant decrease in plasma total thyroxine (TT_4) levels in both male and female offspring 4 days postpartum. The TT_4

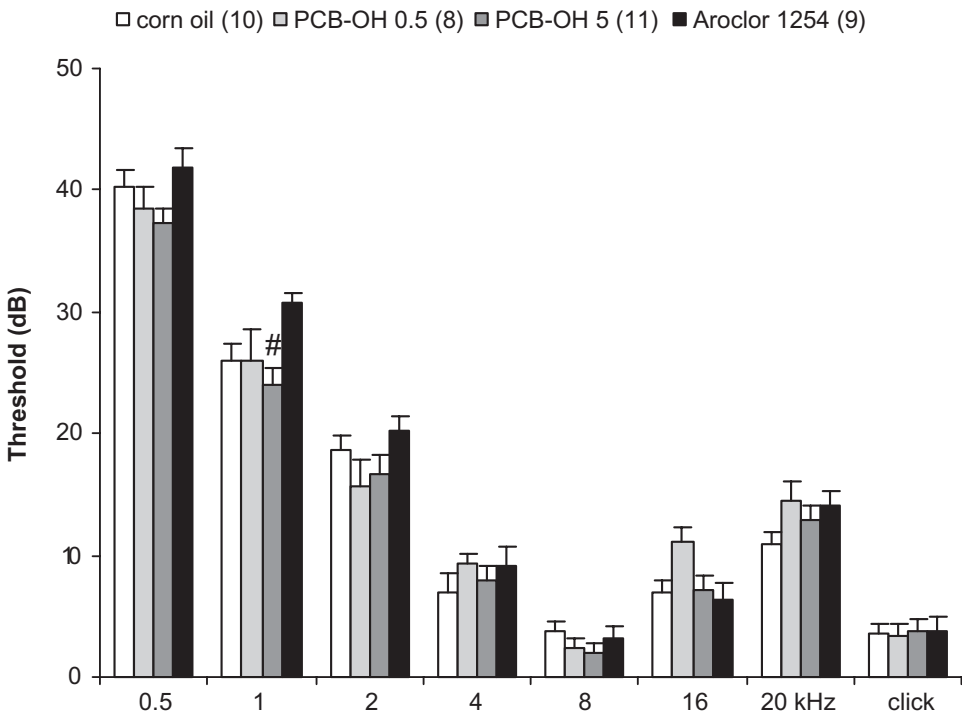

FIG. 8. Auditory thresholds at different tone frequencies in female offspring at PND 300–310 after maternal exposure to corn oil, 4-OH-CB107 (PCB-OH), or Aroclor 1254. # = significantly different from Aroclor 1254, $p < 0.05$. The number of animals from different litters used per treatment group is given in parentheses.

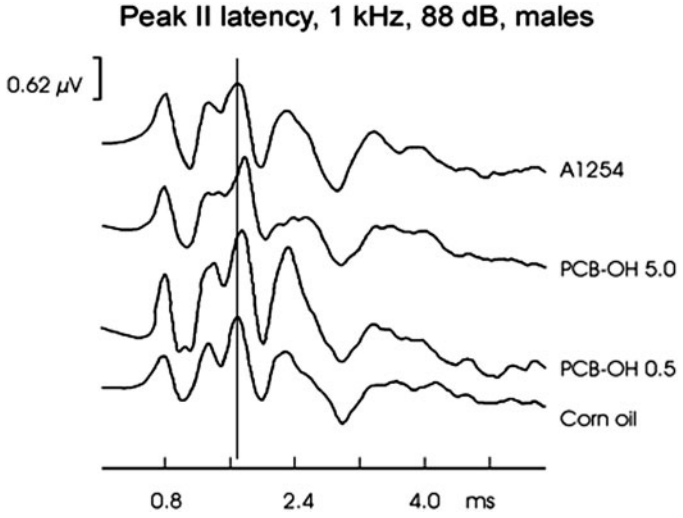

FIG. 9. Grand averages for BAEPs at 1 kHz and 88 dB SPL, abscissa 0.8 ms/div, ordinate 0.620 μV/div, groups from bottom to top, corn oil, PCB-OH 0.5, PCB-OH 5.0, A1254. The stimulus artifact was removed from the traces.

reductions in the 5 mg/kg 4-OH-CB107 exposure group at PND 4 (approximately 34%) were less severe than reductions observed in fetuses at GD 20 (reduction of 89%, Meerts *et al.*, 2002). This phenomenon was also observed after exposure of dams to 25 mg/kg Aroclor 1254 in a similar experimental set-up (Morse *et al.*, 1996a). The fact that Aroclor 1254 exposed offspring showed a more severe reduction in TT_4 levels at PND 4 compared to the 4-OH-CB107 exposed offspring (this study) whereas the reduction in fetal TT_4 levels was lower in Aroclor 1254 exposed compared to 4-OH-CB107 exposed fetuses (Meerts *et al.*, 2004) might be explained by kinetic differences between 4-OH-CB107 and Aroclor 1254. It is very likely that 4-OH-CB107 will be distributed and diluted in growing neonates and eliminated faster than the parent compounds. In addition, in neonates dosed with Aroclor 1254, the production of metabolites including 4-OH-CB107 will continue, giving rise to a more continuous exposure to 4-OH-CB107 compared to 4-OH-CB107 treated offspring.

The observation that serum TSH levels in Aroclor 1254 treated offspring 4 days postpartum do not respond to the reductions in thyroxine (T_4) levels is consistent with earlier findings (Goldey *et al.*, 1995a; Hood *et al.*, 1995; Liu *et al.*, 1995; Morse *et al.*, 1996a). Fetal TSH levels at GD 20 were also not increased after Aroclor 1254 exposure (Morse *et al.*, 1996a). It is hypothesized that PCB congeners and/or their metabolites mimic thyroid hormones (McKinney and Waller, 1994; Rickenbacher *et al.*, 1986) and possibly bind to thyroid hormone receptors in the pituitary, thereby blocking TSH release. In contrast, in fetuses after maternal exposure to 5 mg 4-OH-CB107/kg body weight from GD 10 to GD 16, fetal TSH levels at GD 20 were significantly increased by 124%, most likely as a response to decreased T_4 levels (Meerts *et al.*, 2002). At PND 4 (this study), TSH levels in 4-OH-CB107 treated neonates were

TABLE 1
Latency of Peak II at a Frequency of 1 kHz and Different Sound Pressure Levels (SPL)

Exposure	1 kHz		
SPL (dB)	48	68	88
Female offspring			
Corn oil	1.987 ± 0.047	1.818 ± 0.032	1.774 ± 0.044
4-OH-CB107 (0.5)	2.087 ± 0.049	1.918 ± 0.042*	1.881 ± 0.065
4-OH-CB107 (5)	2.040 ± 0.035	1.838 ± 0.024	1.806 ± 0.043
Aroclor 1254	2.040 ± 0.037	1.866 ± 0.033	1.768 ± 0.025
Male offspring			
Corn oil	1.961 ± 0.028	1.803 ± 0.025	1.737 ± 0.041
4-OH-CB107 (0.5)	1.995 ± 0.047	1.840 ± 0.024	1.739 ± 0.046
4-OH-CB107 (5)	2.088 ± 0.125	1.934 ± 0.115	1.818 ± 0.146
Aroclor 1254	1.986 ± 0.027	1.778 ± 0.020	1.646 ± 0.059

4-OH-CB107 = PCB-OH.
Data are presented as mean ± SE.
*Significantly different from Aroclor, $p < 0.05$.

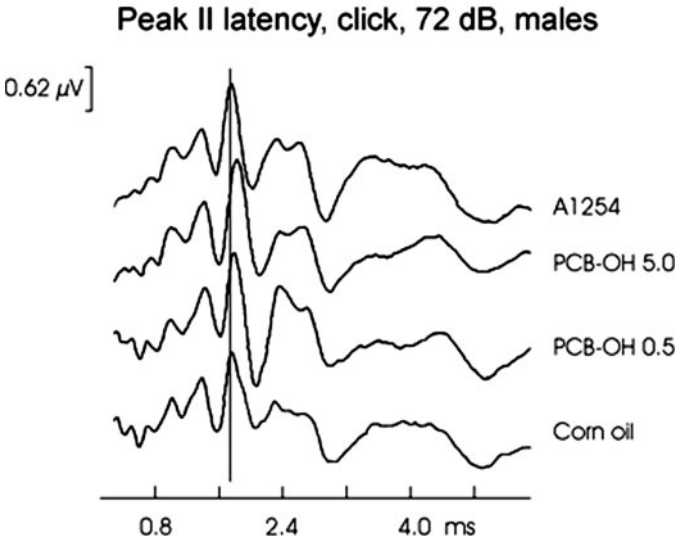

FIG. 10. Grand averages for BAEPs after click stimulation at 72 dB SPL, abscissa 0.8 ms/div, ordinate 0.620 µV/div, groups from bottom to top, corn oil, PCB-OH 0.5, PCB-OH 5.0, A1254. The stimulus artefact was removed from the traces.

comparable to control levels in corn oil treated offspring. Levels of TSH were also unaffected in offspring at the age of 11 months.

The observed increase in locomotor activity in the offspring in the last 3 min of the trial in the open field test indicates an impaired habituation in all exposed groups. Habituation was observed in the control animals, whereas all exposed groups exhibited elevated activity levels in the last 3 min. Increased activity is a well-known effect caused by PCB mixtures, *ortho*-substituted and coplanar congeners in rats (Hany *et al*., 1999b; Holene *et al*., 1995; Jacobson and Jacobson, 1997; Lilienthal *et al*., 1990; Schantz *et al*., 1995). Also in mice, increased locomotor activity has been reported to occur in adult animals after prenatal and postnatal exposure to Aroclor 1254 (Storm *et al*., 1981), or neonatal exposure to coplanar PCBs (Eriksson *et al*., 1991; Eriksson and Fredriksson, 1998) and *ortho*-chlorinated PCBs (Eriksson and Fredriksson, 1996). Agrawal *et al*. (1981) showed that elevated levels of locomotor activity induced by developmental exposure of mice to a high dose of 3,3′,4,4′-tetrachlorobiphenyl was associated with decreased dopamine concentrations in the corpus striatum. In this study, using low to moderate doses of Aroclor 1254 and/or 4-OH-CB107, no significant changes were observed in brain dopamine concentrations in both male and female offspring exposed *in utero*. However, concentrations of the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were slightly decreased in the caudate nucleus from male and female offspring exposed to 5 mg/kg 4-OH-CB107, and slightly increased in the caudate nucleus of Aroclor 1254 exposed offspring, suggesting that both 4-OH-CB107 and Aroclor 1254 are able to exert effects on dopamine metabolism or synthesis. In contrast, Morse *et al*. (1996b) showed only alterations in serotonin metabolism in rat brain after prenatal exposure to Aroclor 1254 in the same experimental set-up.

The passive avoidance data indicate subtle changes in the course of latencies across the three trials that followed the conditioning trial. Latencies were reduced in young adult males of the low-dose group treated with 4-OH-CB107, whereas in older males reductions were found 24 h after conditioning (though not significant). Similar latency decreases were detected in rats prenatally exposed to 2,2′,4,4′-tetraCB and, in particular, 3,3′,4,4′-tetraCB (Weinand-Härer *et al*., 1997). The authors suggested that the PCB effects on thyroid hormone levels might partially explain the observed effects on neurochemical processes and behavior. Iodine-deficient rats also showed a poorer performance in the passive avoidance task, accompanied with reduced levels in T_4 and elevated TSH levels (Overstreet *et al*., 1984). However, the fact that maternal exposure to Aroclor 1254 did not cause alterations in passive avoidance in the present study suggests that mechanisms other than reduced thyroid hormone levels during development mediate long-lasting influences on this neurobehavioral task.

The catalepsy test is a standard pharmacological test to investigate extrapyramidal side effects of neuroleptic compounds due to blocking of dopamine D2 receptors in the neostriatum. In this study, haloperidol-induced catalepsy was used to examine effects on striatal function caused by 4-OH-CB107 or Aroclor 1254. Male rats treated with 4-OH-CB107 or Aroclor 1254 showed decreases in the latencies to movement onset. This suggests alterations in the interaction between the serotonergic and dopaminergic systems, because it is known that catalepsy induced by dopamine receptor antagonists can be completely antagonized by the administration of serotonin receptor agonists

TABLE 2

Biogenic Amine Concentrations (ng/mg tissue, fresh weight) in Different Brain Regions of Male and Female Offspring at the Age of 11 Months, Following *In Utero* Exposure to 4-OH-CB107 or Aroclor 1254 from Gestational Days 10 to 16

	Brain region	Control	4-OH-pentaCB (0.5 mg/kg)	4-OH-pentaCB (5.0 mg/kg)	Aroclor 1254 (25 mg/kg)
Male					
DOPAC	NA	2.63 ± 0.19 (10)	2.25 ± 0.37 (7)	1.66 ± 0.21* (10)	2.50 ± 0.27 (8)
DOPAC	CN	2.27 ± 0.10 (11)	2.24 ± 0.23 (7)	1.86 ± 0.14$^{\#\#}$ (11)	2.70 ± 0.21 (8)
5-HIAA	FC	0.024 ± 0.002 (11)	0.023 ± 0.002$^{\#\#}$ (6)	0.026 ± 0.002$^{\#}$ (10)	0.039 ± 0.004** (8)
5-HIAA	CN	0.020 ± 0.001 (11)	0.027 ± 0.003$^{\#\#}$ (7)	0.021 ± 0.001 (10)	0.018 ± 0.002 (8)
HT	FC	0.012 ± 0.001 (11)	0.011 ± 0.001$^{\#}$ (6)	0.014 ± 0.001 (10)	0.021 ± 0.004* (8)
HVA	PFC	0.033 ± 0.006 (9)	0.043 ± 0.007 (4)	0.024 ± 0.004 (6)	0.023 ± 0.004 (6)
HVA	CN	0.71 ± 0.02 (10)	0.63 ± 0.06 (7)	0.55 ± 0.03* (11)	0.66 ± 0.04 (8)
DOPAC/DA (%)	CN	16.0 ± 1.0 (10)	20.7 ± 1.6 (7)	16.7 ± 0.9$^{\#}$ (11)	22.2±2.0* (8)
Female					
DOPAC	NA	3.20 ± 0.34 (8)	4.21 ± 0.23 (7)	4.16 ± 0.39 (10)	4.32 ± 0.33 (10)
DOPAC	CN	3.25 ± 0.37 (9)	3.41 ± 0.42 (8)	2.83 ± 0.21 (10)	3.76 ± 0.43 (10)
5-HIAA	FC	0.03 7± 0.003 (9)	0.039 ± 0.003 (8)	0.037 ± 0.003 (9)	0.045 ± 0.003 (9)
5-HIAA	CN	0.025 ± 0.001 (9)	0.031 ± 0.001 (7)	0.029 ± 0.002 (9)	0.029 ± 0.002 (9)
HVA	PFC	0.051 ± 0.009 (9)	0.077 ± 0.014 (8)$^{\#}$	0.049 ± 0.005 (9)	0.034 ± 0.005 (10)
HVA	CN	0.73 ± 0.05 (9)	0.76 ± 0.10 (8)	0.61 ± 0.04 (10)	0.67 ± 0.07 (10)
DOPAC/DA (%)	CN	36.4 ± 5.7 (9)	41.2 ± 8.1 (8)	29.0 ± 4.0 (9)	46.0 ± 6.4 (10)

Note. Data are given as mean ± S.E.M.

*Significant difference from control, $p < 0.05$; **$p < 0.01$. $^{\#}$significant difference from Aroclor 1254, $p < 0.05$; $^{\#\#}p < 0.01$. The number of litters per exposure group is given in parentheses.

(Wadenberg, 1996). This is in line with the biogenic amine concentrations measured in the brain of both 4-OH-CB107 treated and Aroclor 1254 treated animals. Effects of developmental exposure to Aroclor 1254 on the concentrations of 5-HT and 5-HIAA are in general accordance with effects found after neonatal hypothyroidism. Savard *et al*. (1984) showed significant increases in 5-HT and 5-HIAA levels in many discrete brain nuclei in the forebrain, midbrain, and hindbrain after neonatal hypothyroidism. Exposure to 4-OH-CB107 resulted in less-pronounced neurochemical effects. However, the observed decreases in latencies of movement onset in the catalepsy test (this study) indicate that neurotransmitter functions may have been influenced after 4-OH-CB107 exposure, aside from post mortem concentrations of neurotransmitters. The observed differences between males (late latency decreases) and females (early latency increases) may be due to differences in kinetics of haloperidol in both sexes, with males showing an earlier onset of catalepsy than females, resulting in more rapid expression and decay of the response.

The precise mechanism by which Aroclor 1254 or the PCB metabolite alter concentrations of neurotransmitters is unknown. 4-OH-CB107 is known to exert anti-estrogenicity *in vitro* (Moore *et al*., 1997; Meerts, unpublished results). High concentrations of this metabolite in the developing brain may influence CNS dopaminergic and serotonergic function, as there appear to exist interactive relationships between estrogens and DA as well as between estrogens and 5-HT (Rubinow *et al*., 1998; reviewed in McEwen and Alves, 1999). Another explanation might be the recently reported extremely potent inhibition of human estrogen sulfotransferase activity (*in vitro*) by environmentally relevant hydroxylated PCBs (Kester *et al*., 2000). The authors showed that 4-OH-CB107 was one of the strongest of the 32 tested compounds, with an IC_{50} of 0.15–0.25 nM. This suggests that 4-OH-CB107 might indirectly induce estrogenic activity by increasing estradiol bioavailability in target tissues.

The effects on auditory thresholds in Aroclor 1254 treated offspring are visible only in the low-frequency range (500 Hz to 4 kHz). This is in line with effects observed so far concerning the influence of thyroid hormone deficiencies on auditory thresholds (Goldey *et al*., 1995a). In addition, the results shown for Aroclor 1254 treated animals are in line with results presented by Goldey *et al*. (1995b) and were related to hair cell loss in the apical part of the cochlea (Crofton *et al*. 2000a). Animals treated with 4-OH-CB107 showed no increase in BAEP thresholds, suggesting that this metabolite exerts no deleterious effects on the cochlea. However, the slight prolongation of latencies in metabolite-exposed groups may indicate effects on the neural part of the auditory system. Alternatively, this may be explained by the observations of Crofton *et al*. (2000b), who showed in cross-fostering studies that lactational exposure to Aroclor (postnatally) is the major cause of ototoxicity.

In conclusion, maternal exposure to the PCB metabolite 4-OH-CB107 can exert adverse effects on neurotransmitter levels and brain development in rat offspring, that are both similar to and somewhat different from the effects observed after Aroclor 1254 exposure.

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